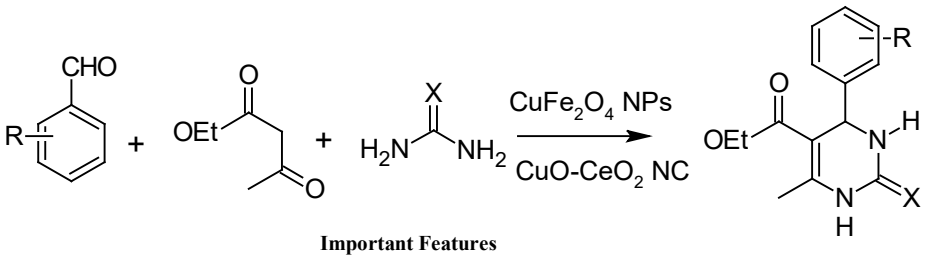
Supporting Information

Ecofriendly Synthesis of DHPMs using Copper-based Nano catalysts and Evaluation of Antibacterial Activity

Khushboo Sharma, Narsingh Khatik, Abhinav Raj Khandelwal, Ravina Meena and Seema Bhadauria, Malvika Singh and Harshita Sachdeva*



Important Features
1. Microwave irradiation
2. Short reaction time and excellent product yield
3. Experimental simplicity and easy work up

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1. **General Information.** Chemicals used for the reaction were purchased from sigma Aldrich and Merck and were not purified. TLC was used to check the progress of reaction using Benzene: Ethyl acetate (8:2) as eluent. Melting point apparatus was used for the determination of melting points. The room temperature means 30–40°C. The resulting compounds were recognised based on their melting points from published sources and their spectral (1H NMR and IR) data. Nanoparticles were characterized by PXRD with a PANalytical X'Pert Pro Diffractometer using Cu (Kα) radiation (wavelength: 1.5406 Å), operated at 45 kV and 40 mA at room temperature in the range of 2θ from 20.0084 to 89.9804. Infra-red spectra were recorded in KBr on a Perkin Elmer Infrared RXI FTIR spectrophotometer. Reactions were conducted in a Catalyst Systems Scientific Multimode MW oven running at 700 W and producing 2450 MHz frequency.
2. **Synthetic Procedures**

Synthesis of $CuFe_2O_4$ NPs. [1] 100 mL solution (0.1M) each of $CuCl_2.2H_2O$ and $FeCl_3$ were prepared separately and were mixed together vigorously under ultrasonication for 30 min. at room temperature. In order to maintain a pH of 9 for precipitation, 6N NaOH solution was

added drop wise to the homogeneous mixture. Co-precipitation was achieved after 2 hours, and the co-precipitated particles were vigorously agitated for an additional 2 hours. In order to balance the pH and remove excess ions, the residue was repeatedly rinsed with deionized distilled water and propanol after the co-precipitated particles had been filtered using the vacuum filtration process. $CuFe_2O_4$ NPs were dried at 80 °C for 24 hours in a hot electric oven and calcined at 600 °C for 6 hours in a muffle furnace.

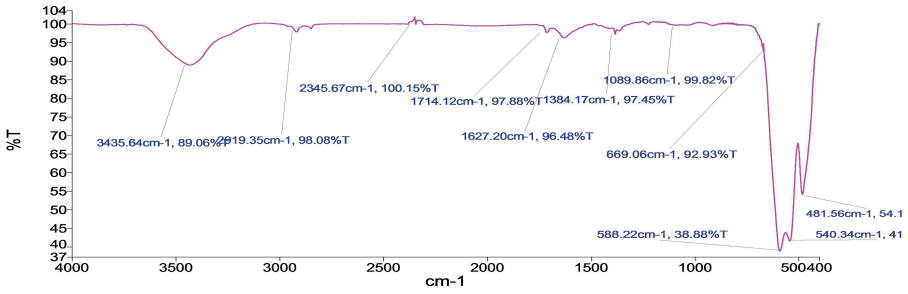


Figure 1. FT-IR spectrum of copper ferrite nanoparticles

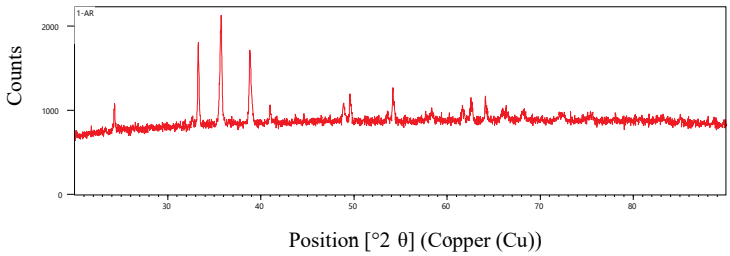
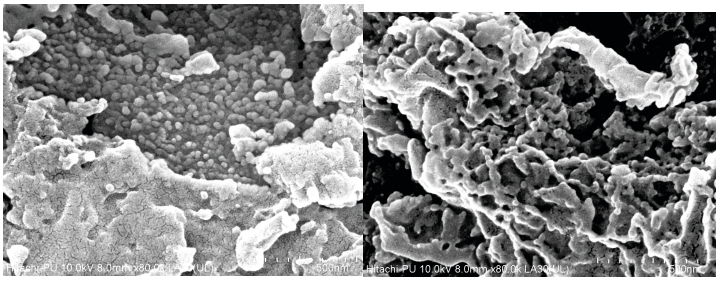


Figure 2. X-ray diffraction pattern of $CuFe_2O_4$ nanoparticles



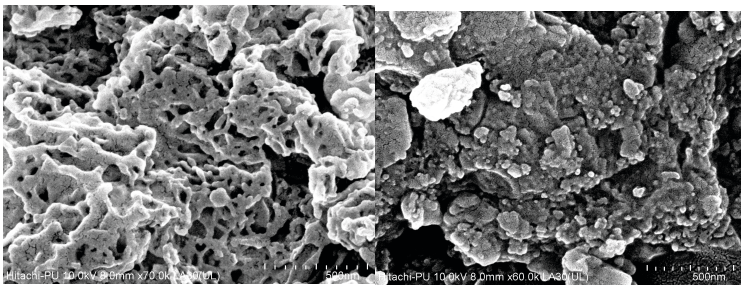


Figure 3. SEM images of $CuFe_2O_4$ nanoparticles

Synthesis of $CuO\text{-}CeO_2$ nanocomposite catalysts: [2] 100 mL (0.1 M) aqueous solution each of Cu $(NO_3)_2.3H_2O$ and Ce $(NO_3)_3.6H_2O$ were stirred at room temperature. It is demonstrated that the molar ratio of starting materials dictates the size of particles [3, 4]. After repetition of process of maintaining 6, 7, 8, and 9 pH (by adding 6 M aqueous NaOH solution), it is observed that more precipitation occurs at pH 9. Hence, 6 M aqueous solution of NaOH was quickly added to the mixture for maintaining pH 9. The complete precipitation occurs after 6 hrs of continuous stirring at room temperature. After filtration and washing of the precipitate, it was kept overnight in an oven at 60°C for drying. Then, the dried precipitate was powdered and irradiated in microwave oven for 6 minutes. This led to the formation of small-sized and uniform nanoparticles.

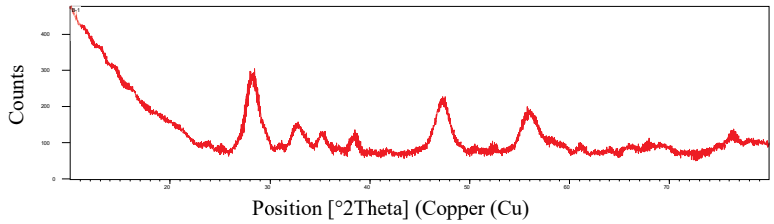


Figure 4. X-Ray diffraction pattern of $CuO\text{-}CeO_2$ nanocomposite

General Procedure for the Synthesis of Ethyl-6-methyl-2-oxo/thioxo-4-(substituted-phenyl)-1, 2, 3, 4-tetrahydropyrimidin-5-carboxylate (4). The compounds were synthesized by the following methods: **Method I.** A mixture of an aromatic aldehyde (1 mmol), ethylacetoacetate (1 mmol), urea/thiourea (2 mmol), and $CuFe_2O_4$ NPs (0.3 m mol) in absolute ethanol (10 mL) was exposed to microwave radiation at 245 Watts for 8-10 minutes. TLC was used to monitor the reaction's progress. After the reaction was finished, the catalyst was magnetically recovered using an external magnet. In order to obtain the pure product, the reaction mixture was cooled to

room temperature, poured onto crushed ice, filtered, and recrystallized using either ethanol or an ethyl acetate and petroleum ether (1:1) mixture.

Method II. In a microwave oven, a mixture containing an aromatic aldehyde (10 mmol), ethylacetoacetate (10 mmol), urea (20 mmol), and CuO-CeO_2 nanocomposite (30 mg) in absolute ethanol was charged into glass microwave vessel and refluxed for 5–6 minutes under microwave irradiation at 245 watts. TLC was used to monitor reaction's progress. The catalyst was extracted from the reaction mixture by simple filtration after the reaction was finished. After being cooled to room (30-40 ˚C) temperature, the product was crystallized again from ethanol.

Method III. A mixture of an aromatic aldehyde (10 mmol), ethylacetoacetate (10 mmol), urea (20 mmol), and CuO-CeO_2 NC (0.3 mmol) was added to the RB flask and magnetically stirred at 50 °C for the time required to complete the reaction (as indicated by TLC). The reaction mixture solidifies within 25 to 30 minutes. TLC was used to monitor the reaction's progress. As soon as the reaction was completed, distilled water was added to the mixture and then the reaction mixture was poured onto crushed ice; solid product containing catalyst obtained was filtered and dried under room temperature. The ethanol was then added to the solid product, heat it on water bath till it dissolves in ethanol and then the catalyst was separated by simple filtration. The product obtained was finally recovered from the ethanol and recrystallized to get the pure product.

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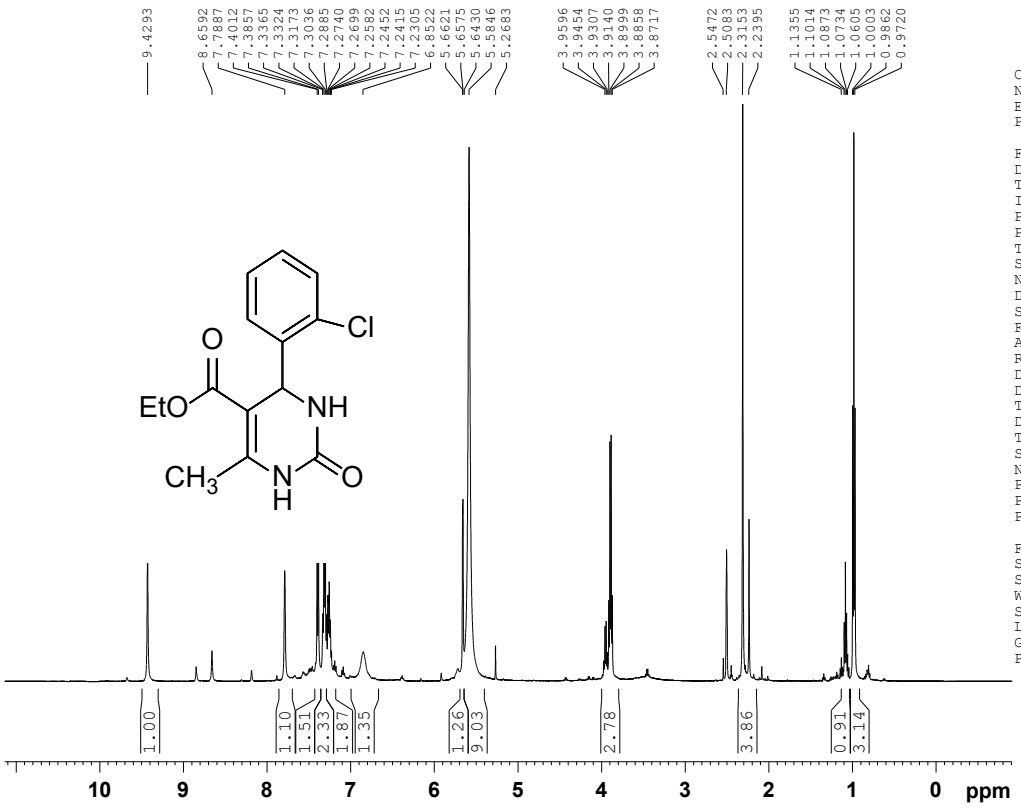

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SAIF, P.U.

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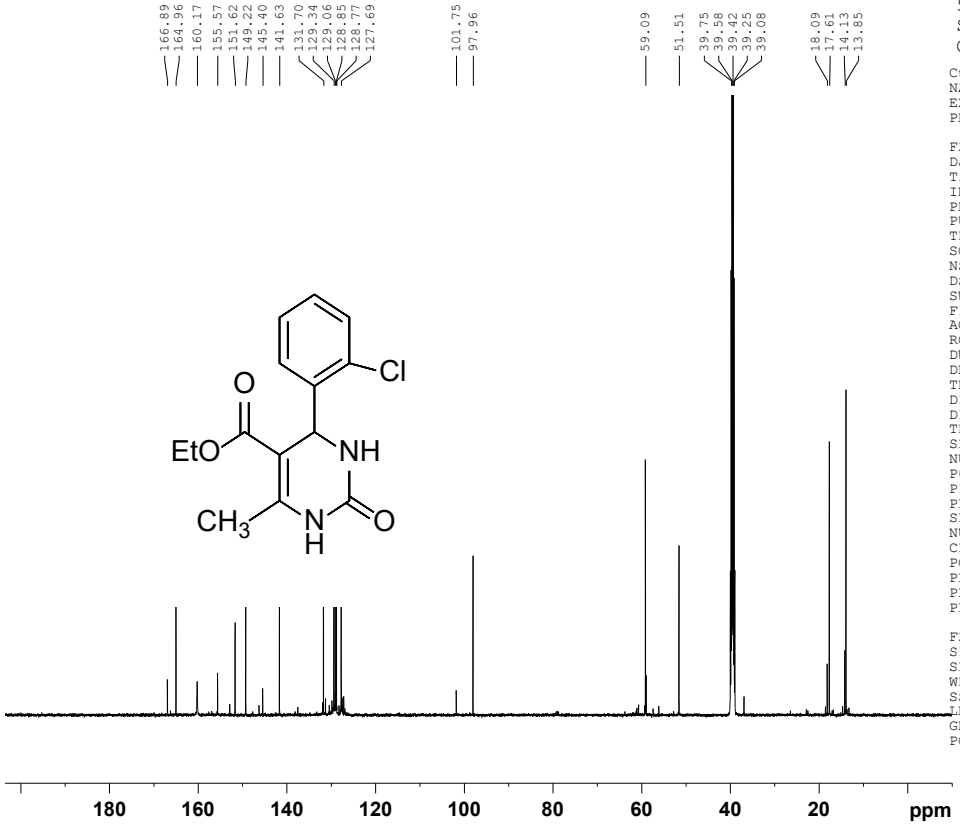

BRUKER
AVANCE NEO
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SAIF, PANJAB UNIVERSITY,
CHANDIGARH

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PROCNO	1

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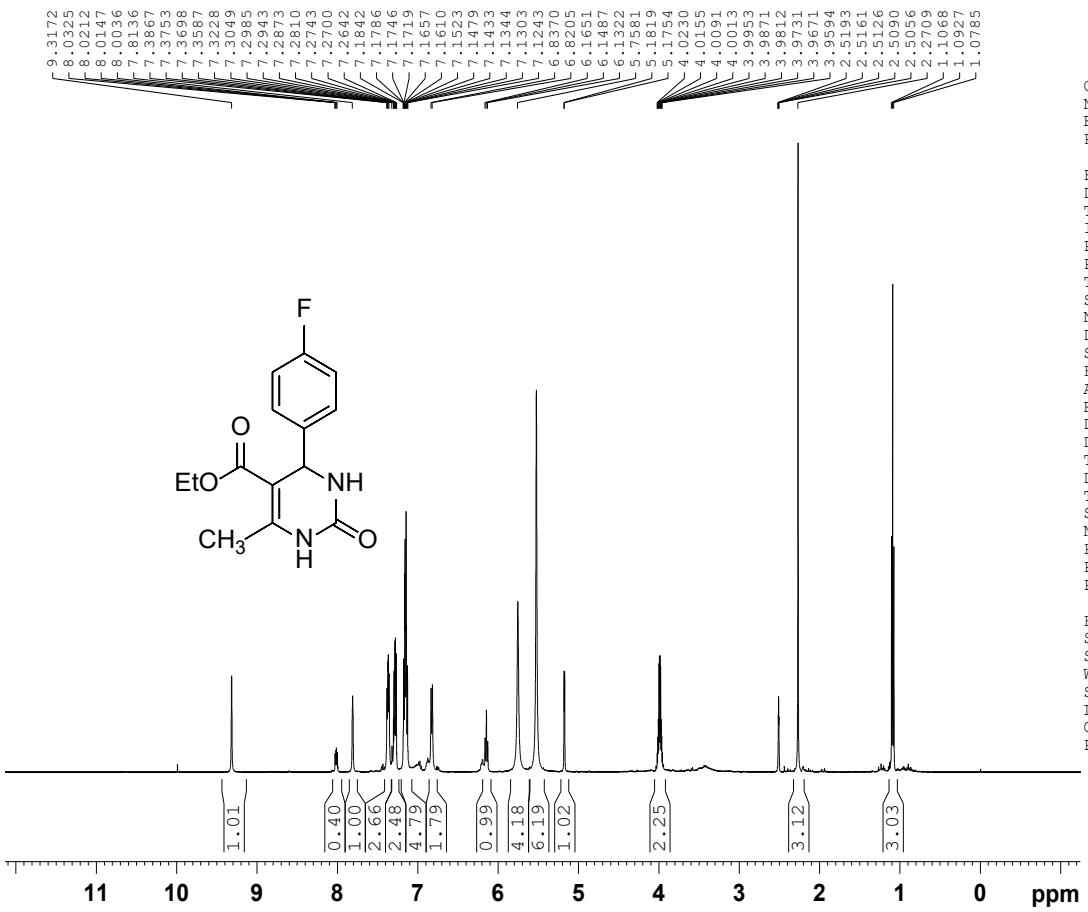

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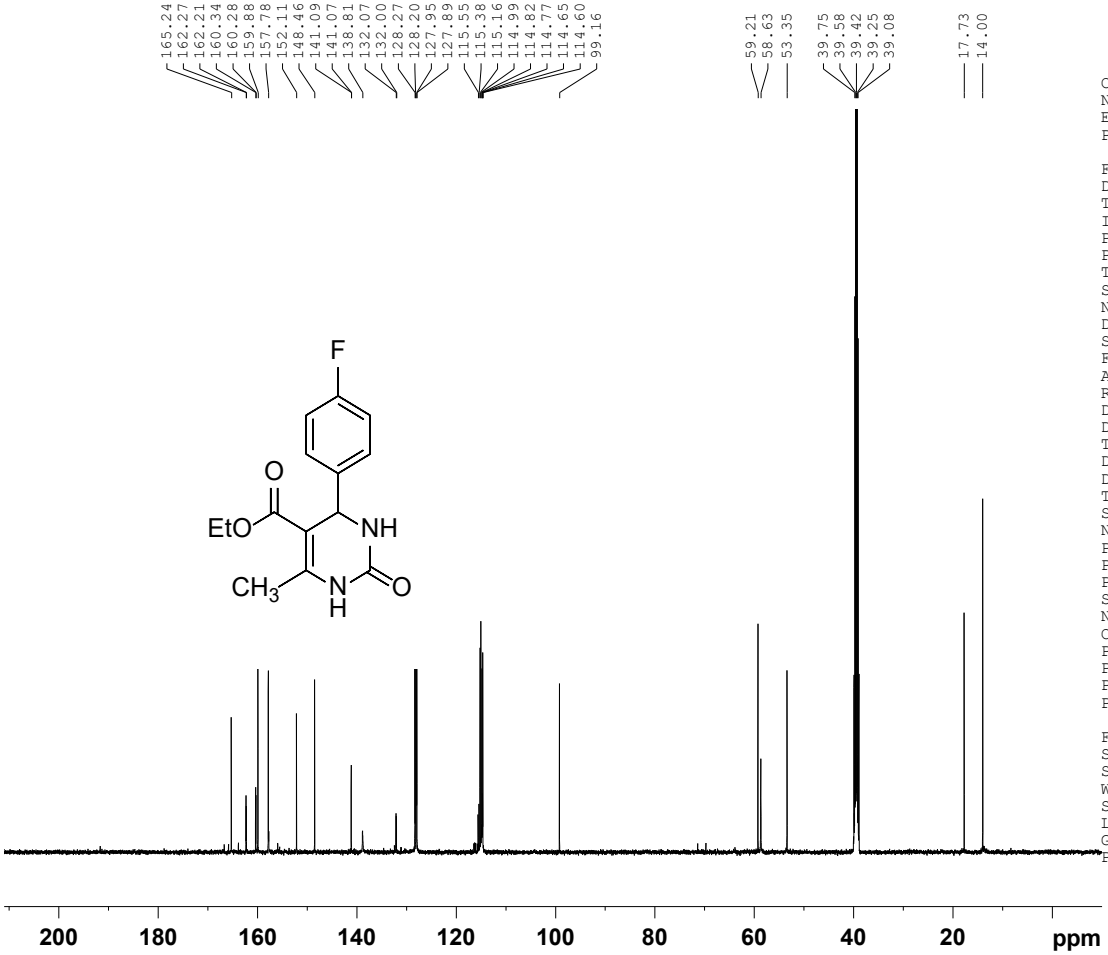

BRUKER
AVANCE NEO
500 MHz NMR SPECTROMETER
SAIF, PANJAB UNIVERSITY,
CHANDIGARH

Current Data Parameters

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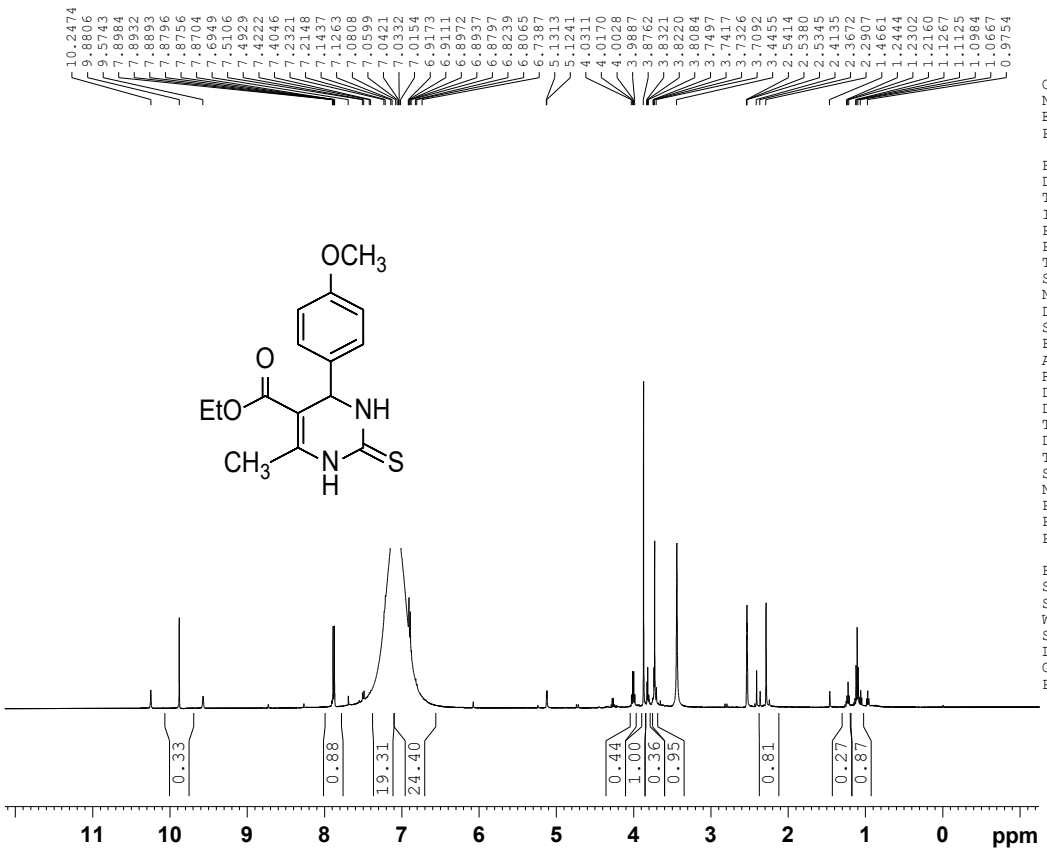

BRUKER
AVANCE NEO
500 MHz NMR
SPECTROMETER
SAIF, P.U.

Current Data Parameters

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PLW1	20.93000031 W

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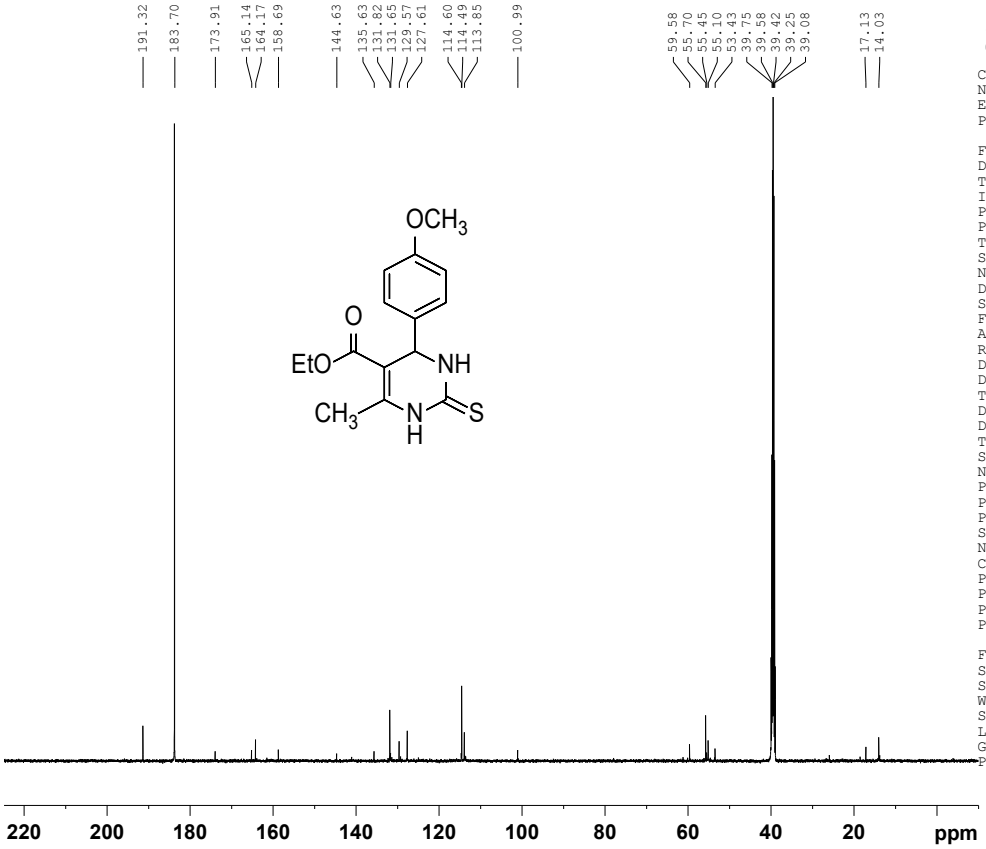

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SAIF, PANJAB UNIVERSITY,
CHANDIGARH

Current Data Parameters

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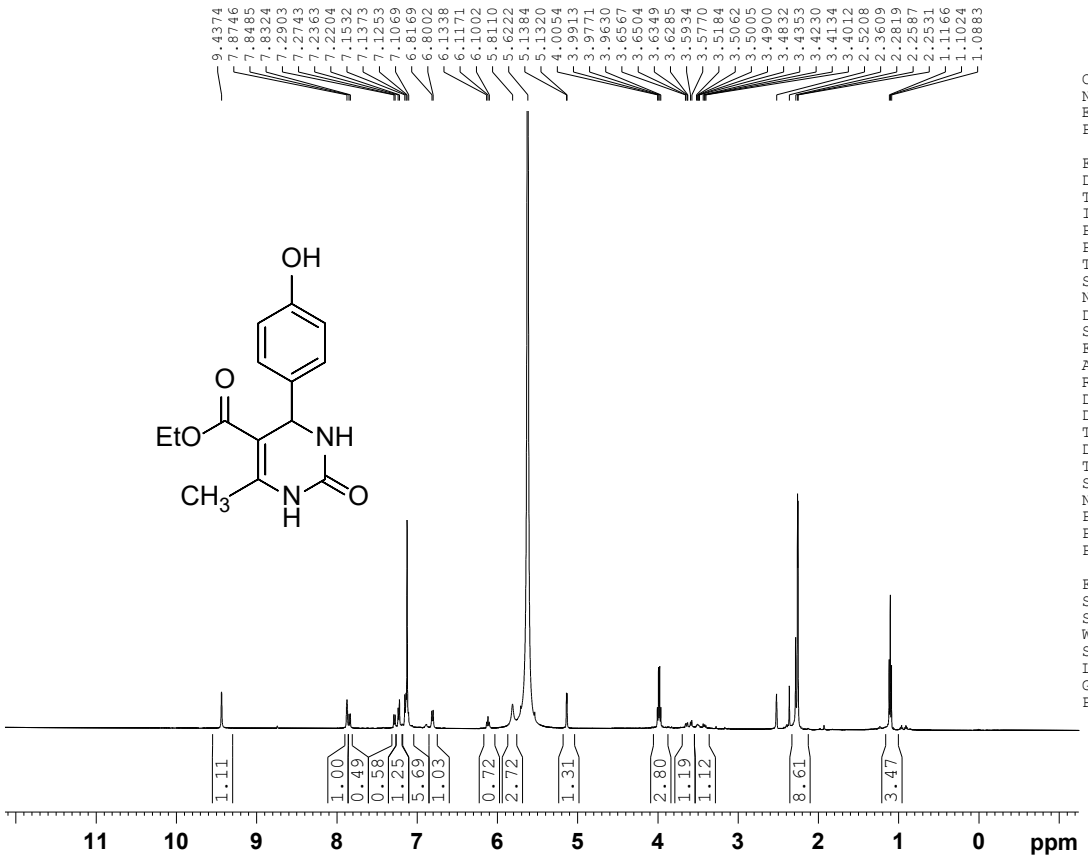

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SAIF, P.U.

Current Data Parameters

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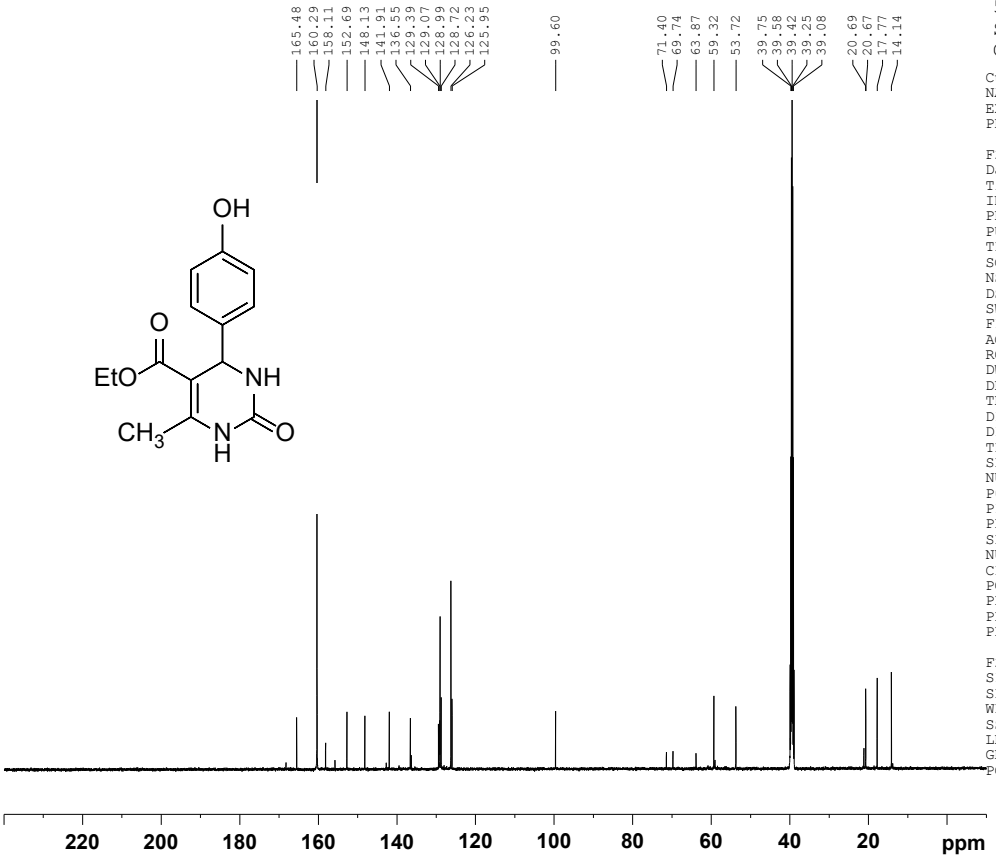

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SAIF, PANJAB UNIVERSITY,
CHANDIGARH

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P0	3.33 usec
P1	10.00 usec
PLW1	83.14099884 W
SFO2	500.1720007 MHz
NUC2	1H
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PCPD2	80.00 usec
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3. Antimicrobial activity: The antimicrobial potential of the given compounds was determined by the standard Agar Disc Diffusion technique (Gould and Bowie, 1952) [6] against the four bacteria. Selected bacterial strains are as follows: *Escherichia coli*, *Bacillus subtilis*, *Bacillus megaterium, and Proteus vulgaris.*

Disc diffusion method: Disc diffusion method was used for the antibacterial screening of the synthesized compounds (Gould and Bowie, 1952) [5] **(Table 4).** In this method, sterilization of standard Whatman filter paper discs of standard size (6.0 mm in diameter) was done at 140°C in an oven for one hour after being soaked with the extract and air dried at room temperature for the removal of any residual solvent that might interfere with the determination. After the test bacteria had been injected into the Nutrient Agar medium, the discs were placed on its surface and air dried to remove any surface moisture. The standard disc (Streptomycin) was placed in each petriplate as a control, and the thickness of the agar medium was maintained uniformly throughout all of the plates. The plates were then incubated at 37°C for 20–24 hours, allowing for easy measurement of the zone of inhibition or decreased growth. Filter paper disc's (6 mm) diameter is included in the inhibition zone. Each sample was examined in triplicate, and for each, an activity index was computed. **Figure 8** shows images of antimicrobial activity of following compounds by Disc Diffusion Method against (i) *Escherichia coli* (ii) *Bacillus subtilis* (iii) *Bacillus megaterium* (iv)*Proteus vulgaris*

$$\text{Activity Index (A.I.)} = \frac{\text{Inhibition Zone (I.Z.) of the Sample}}{\text{Inhibition Zone (I.Z.) of the Standard}}$$

Observation and Results: On one or more of the test bacteria, various test compounds exhibited growth-inhibitory activity **(Table 4).** The activity index was derived by comparing the inhibition zones produced by the test compounds with the inhibition zones produced by the standard. Among all the test compounds, compound 4c responded favorably to all test bacteria other than Escherichia coli while compound 4h responded negatively to all bacteria.

$$\text{Activity Index (A.I.)} = \frac{\text{Inhibition Zone (I.Z.) of the Sample}}{\text{Inhibition Zone (I.Z.) of the Standard}}$$

I.Z. = Inhibition Zone, A.I. = Activity Zone

.**Agar Well Diffusion method:** Compounds were also tested for antimicrobial activity using the agar well diffusion technique on Nutrient Agar plates. The test bacteria were lawn grown on nutrient agar plates. Using a sterile tip, 6 mm wells were bored into the infected medium. It was poured the specified compound into each well. As a positive control, streptomycin was also administered to one well (Standard). It was incubated for 24 hours at 37°C after being allowed to diffuse for about 30 minutes at room temperature. After incubation, the test compounds' antimicrobial activity was determined by looking at the plates for the development of a clear zone around the well. A millimetre measurement of the inhibitory zone (I.Z.) was taken. Triplicates of each sample were evaluated, and the activity index (A.I.) was calculated for each of them **(Table 5).**

Observation and Results: A number of the test microorganisms were inhibited from growing by several test compounds **(Table 5).** The activity index was derived by comparing the inhibition zones produced by the test compounds with the inhibition zones produced by the standard. The agar well diffusion approach produced similar findings. Out of all the test compounds, compound **4c** responded favourably to every test bacterium except Escherichia coli, while compound **4h** responded negatively to nearly every test bacterium.

Activity Index (A.I.) = $\frac{\text{Inhibition Zone (I.Z.) of the Sample}}{\text{Inhibition Zone (I.Z.) of the Standard}}$

I.Z. = Inhibition Zone, A.I. = Activity Zone

Table 4: Antibacterial activity of five test compounds by Disc Diffusion Method

Test Bacteria	Inhibition zone (mm) of Standard (Streptomycin)	Test Compounds									
		4l		**4j**		**4c**		**4a**		**4h**	
		I.Z. (mm)	**A.I.**	**I.Z. (mm)**	**A.I.**	**I.Z. (mm)**	**A.I.**	**I.Z. (mm)**	**A.I.**	**I.Z. (mm)**	**A.I.**
Escherichia coli	27.66	-ve	-	-ve	-	-ve	-	-ve	-	-ve	-
Bacillus subtilis	39.66	-ve	-	-ve	-	12.33	0.31	9	0.22	-ve	-
Bacillus megaterium	25	12.33	0.49	12.66	0.50	9.33	0.37	-ve	-	-ve	-
Proteus vulgaris	26	12.66	0.49	14	0.53	9.66	0.37	9	0.35	-ve	-

Table 5: Antibacterial activity of five test compounds by Agar Well Diffusion Method

Test Bacteria	Inhibition zone (mm) of Standard (Streptomycin)	Test Compounds									
		4l		**4j**		**4c**		**4a**		**4h**	
		I.Z. (mm)	**A.I.**	**I.Z. (mm)**	**A.I.**	**I.Z. (mm)**	**A.I.**	**I.Z. (mm)**	**A.I.**	**I.Z. (mm)**	**A.I.**
Escherichia Coli	39	-ve	-	-ve	-	-ve	-	-ve	-	-ve	-
Bacillus Subtilis	46	-ve	-	-ve	-	14.66	0.31	14.66	0.31	-ve	-
Bacillus Megaterium	47	29	0.62	25	0.53	13.66	0.29	-ve	-	-ve	-
Proteus vulgaris	39	25	0.64	30	0.77	17.33	0.44	13	0.33	-ve	-

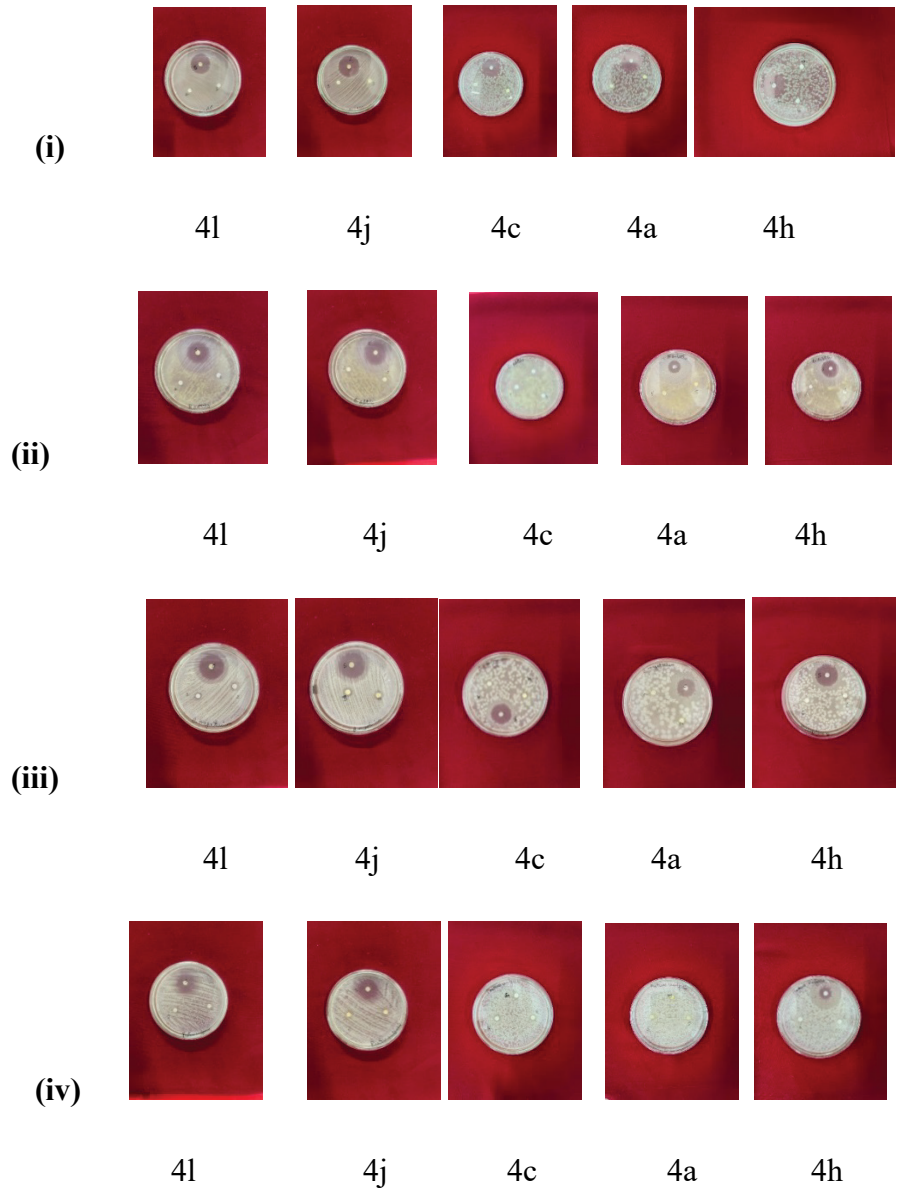

Figure 8. Images of antimicrobial activity of following compounds by Disc Diffusion Method against (i) *Escherichia coli* (ii) *Bacillus subtilis* (iii) *Bacillus megaterium* (iv)*Proteus vulgaris*

References

1. Mondal B, Kundu M, Mandal SP, Saha R, Roy UK, Roychowdhury A, Das D. (2019) Sonochemically synthesized spin-canted $CuFe_2O_4$ nanoparticles for heterogeneous green catalytic click chemistry. ACS Omega. 4(9):13845-52.
2. Albadi J, Mansournezhad A (2013) CuO-CeO_2 nanocomposite: A green recyclable catalyst for the synthesis of 3, 4-dihydropyrimidin-2 (1H)-ones/thiones. Iran. J. Catal. 3(2):73-7.
3. Bae DS, Han KS, Adair J H (2002) J. Mater. Chem. 12:3117-3120.
4. Katsuki H, Shiraishi A, Komarneni S, Moon WJ, Toh S, Kaneko K (2004) J. Ceram. S. Japan 112:384-387.
5. Gould J C and Bowie J H (1952) The determination of bacterial sensitivity to antibiotics Edinb. Med. J. 59:178.